iGEM 2016 – Microbiology – BMB – SDU

Project type: Bacteriocin **Project title:** Cloning composite part into iGEM standard plasmid. **Sub project:** Insertion of k2018010 (Laterosporulin) into pSB1C3 plasmid	**Creation date:** 2016.08.08 **Written by:** Astrid Sophie Pejstrup Honoré **Performed by:** Pernille Vigsø Rasmussen, Brian Kenn Baltzar, Astrid Sophie Pejstrup Honoré, Cathrine Høyer Christensen

1. SOPs in use.

SOP number: SOP0007_v01 LA plates with antibiotic

SOP number: SOP0022_v01 Competent cell - freeze-stock

SOP number: SOP0023_v01 Ca^{++} transformation

SOP number: SOP0009_v01 TSB transformation

Plasmid purification kit

SOP number: SOP0001_v01 ON culture of *E.coli*

SOP number: SOP0004_v01 bacterial freeze stock

SOP number: SOP0017_v01 Fast digest

SOP number: SOP0015_v01 Ligation

Gel purification kit

SOP number: SOP0021_v01 Colony PCR with MyTaq

2. Purpose.

To insert composite part: k2018010 (Laterosporulin), into iGEM standard plasmid; pSB1C3.

3. Overview.

Day	SOPs	Experiments
1	SOP0023_v01	Ca^{++} transformation
2	SOP0001_v01	ON culture of *E.coli*
3	Miniprep kit	Plasmid purification
3	SOP0004_v01	Bacterial freeze stock
4	SOP0017_v01	Fast digest
5	SOP0017_v01	Fast digest
5	Gel purification kit	Gel purification
6	SOP0015_v01	Ligation
7	SOP0009_v01	TSB Transformation
8	SOP0021_v01	Colony PCR with MyTaq
8	SOP0001_v01	ON culture of *E.coli*
9	Gel purification kit	Gel purification
9	SOP0004_v01	Bacterial freeze stock

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Appropriate medium ex. LB	1% Tryptone 1% NaCl 0.5% Yeast extract	Oxoid Sigma-Aldrich Merck	Media lab or V18-40 5-0	
Glycerol	50 %	AppliChem	Anne Mette, RT	
LB		Anne-Mette		
LA	1% Tryptone 1% NaCl 0.5% Yeast extract 1.5% agar	Oxoid Sigma-Aldrich Merck Difco agar from BD	Anne-Mette Or	

			V18-405-0	
Water	Demineralised milli-Q autoclaved water	Milli-Q water purification system (Millipore)	RT	Water
MyTaq™HS Red Mix	http://www.bioline.com/documents/product_inserts/MyTaq%E2%84%A2%20HS%20Red%20Mix.pdf#zoom=130	Bioline	V18-405a-2	
Reverse primer	Made specific to the template	Sigma-Aldrich		
Forward primer	Made specific to the template	Sigma-Aldrich		
Ligasebuffer		Agilent Technologies	Freezer at 1. Floor	
Ligase			Freezer 1. Floor	Ligase
FastDigest enzyme		Agilent Technologies	Freezer at 1. Floor	
Fast digest green / 10 x FastDigest Buffer		Agilent Technologies	Freezer at 1.	
$CaCl_2$	0.1M		Chem room	
$MgCl_2$	0.1M		Chem room	$MgCl_2$
liquid nitrogen	liquid nitrogen	liquid nitrogen	liquid nitrogen	
Fast digest green		Agilent Technologies	Freezer at 1.	
6x DNA Loading Dye		GeneRuler	fridge floor 1	
Fort. LB		the new Anne-Mette	Autoclave room	
Polyethylene glycol (PEG) 3.350		Sigma Aldrich	Micro Chemical room	

Dimethylsulfoxid (DMSO)		Sigma Aldrich	Micro Chemical room
Magnesiumchloride (MgCl2) 1M	1M	The New Anne-Mette	Autoclave

5. Other

As competent cells, LB and LA media was used by all parts of our project and not just this protocol the dates for use of these SOPs are not added. this comment deal with SOP number: SOP0007_v01 and SOP0022_v01

Gel Electrophoresis is set at 75 V for 30-45 minutes, dependent on the gel percent.

6. Experiment history.

Date (YY.MM.DD)	**SOPs**	**Alterations to SOPs and remarks to experiments**
16.07.14		resuspended the received 4µg of plasmid with 40µl dH2O
16.07.14	SOP0023_v01 Ca++ transformation	Their where used 2.5 µl instead of the normal 1µl
16.07.16	SOP0001_v01 ON culture of *E. coli*	
16.07.17	miniprep kit	50 µl were used for elution step
16.07.17	SOP0004_v01	Name; #30
16.07.19	SOP0017_v01 Fast digest	Fast digest enzyme(pst1) cut overnight
16.07.20	SOP0017_v01 fast digest	Fast digest enzymes(EcoR1) cut in 30 min.
16.07.20	Gel electrophoresis	1kb plus DNA ladder(Green) was used

16.07.20	Gel purification kit	30 µl were used for elution step
16.07.21	SOP0015_v01 ligation	At the ligation step following ratios of vector and DNA were used: 1:1 and 1:5. For cPCR most cultures are taken from both ratio, and 3 replicates, to minimise the risk of getting religation.

Ratio	**1:1**	**1:5**
Ligase buffer	2µl	2µl
Ligase	1µl	1µl
BG51	1µl	5µl
PG17	1µl	1µl
H_2O	16µl	11µl

16.07.22	SOP0009_v01 TSB transformation	5 µL plasmid is used for every sample
16.07.24	SOP0021_v01 cPCR with MyTaq	Made on 3 colonies.

Segement	Step	Temperature	Duration
1	Initial denaturation	98 °C	2 min
2	34 cycles	98 °C	10 sec
		72 °C	15 sec
		72 °C	15 sec
3	Final extension	72 °C	5 min
4	Keep the sample	12 °C	HOLD

	cold		

16.07.24	SOP0001_v01 ON culture of *E. coli*	
16.07.25	Gel electrophoresis on cPCR product	1 kb DNA ladder(green) was used
16.07.25	miniprep kit	
16.07.25	Freeze stock	Name; #47

7. Sample specification.

Sample name	Sample content	From	Used for / Saved where
BR37	pUCIDT-AMP:k2018010	IDT	transferred to *E. coli* / saved in coolbox
BR38	pUCIDT-AMP:k2018010	IDT	Purifeid BR37, used to Cut with restriction enzymes and ligated with the vector/saved in coolbox and on Freeze stock (#30)
PR17	pSB1C3:k748002	iGEM	Vector cut with pst1 and EcoR1 / plastic cool box
BG51	k2018010	IDT	Cuted BR38 with pst1 and EcoR1/ Saved in coolbox
BB54	pSB1C3:k2018010		vector and composite part ligated and transferred to *E.coli.* /Saved in coolbox
BR64	pSB1C3:k2018010		purified and transferred BB54 /

freeze stock in *E.coli* (#47)

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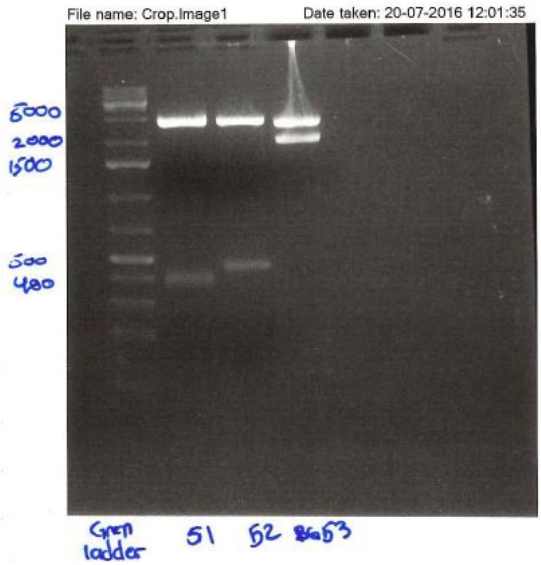
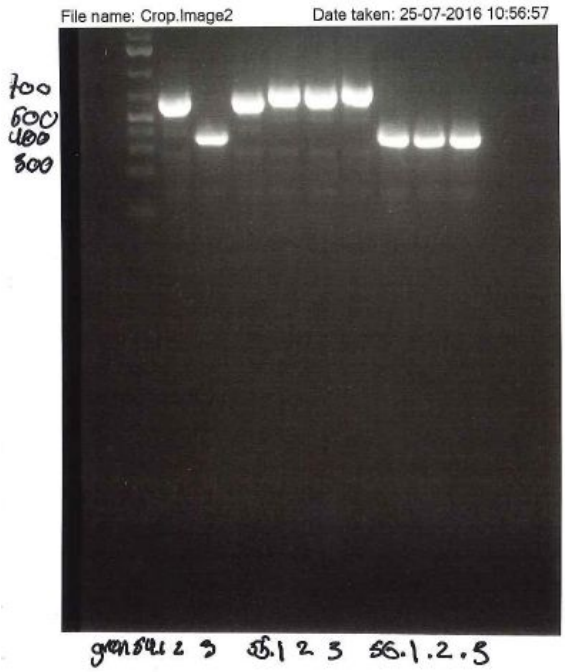
8. Remarks on setup.

9. Results and conclusions.

The Gel Photo below shows the results from cutting the BR38 with Pst1 overnight and EcoR1 at 30 minutes, and after that named BG51 (At the photo named; 51). The composite part is 395 bp. It is possible from the Gel photo to assume that the DNA is cut correctly. And from this gel is the BG51 band with around 400 bp purified, for a ligation with PR17, the vector.



The Gel Photo under this, shows from the left the ladder; in this gel is used Green ladder. the following bands are BB54, 1, 2 and 4 there is the ligation of the vector PR17 and the insert BG51. From the photo it can be expect that sample 54.1 and 54.3 are the vector with insert, and 54.2 is a religation.



10. Appendixes